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(71)(72) Applicants and Inventors: SLEAT, Robert [GB/GB]; 38 Nottingham Street, Canton, Cardiff CF5 1JP (GB). BEWLEY, Richard, John, Foster [GB/GB]; 15 Bittern Way, Lavernock Park, South Glamorgan CF6 2FS (GB). VON DER DICK, Hans [DE/CA]; 3315 Upton Place NW, Calgary, Alberta T2N 4G9 (CA).

(74) Agents: WOODS, Geoffrey, Corlett et al.; J.A. Kemp & Co., 14 South Square, Gray's Inn, London WC1R 5LX (GB).

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(57) Abstract

A method of assaying a plurality of soil samples collected at intervals across an exploration territory for subsurface oil or gas deposits, which method comprises: (i) determining from a first portion of a first sample a first value which represents a first microbial activity and which is attributable to microorganisms which metabolise a selected hydrocarbon gas; (ii) determining from a second portion of the first sample a second value which represents a second microbial activity and which is attributable to a representative proportion of all microorganisms in the sample; (iii) repeating steps (i) and (ii) for further samples; and (iv) determining for each sample the first microbial activity relative to the second microbial activity from the said first and second values obtained for each sample. The concentration of free hydrocarbon gas may also be taken at each site at which a soil sample is taken and combined with the determination made in step (iv).

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OIL AND GAS EXPLORATION

This invention relates to methods for use in surface geochemical prospecting for subsurface hydrocarbon oil or gas deposits, conventionally explored by using geological and seismic methods.

It has been known for quite some time that subsurface hydrocarbon gases can seep upwards from subsurface oil and gas deposits. The four major fates of the subsurface hydrocarbon gases in near surface soils are 1. presence as free gas, 2. oxidation to carbon dioxide or intermediate products by microorganisms, 3. adsorption onto soil particles and 4. dissolution into associated aqueous phase (ground water). The presence of hydrocarbon gases (even in low concentrations) in the surface soils may be therefore indicative of underlying hydrocarbon-bearing strata. It has further been known that if soils are exposed for prolonged periods to hydrocarbon gases, specific microbial populations may develop which feed from the gases concerned.

It has been proposed before to explore for subsurface oil and gas deposits through examination of surface soils. Previous microbial techniques have depended upon measuring hydrocarbon-oxidising activities in the soil or isolating specific hydrocarbon-oxidising microorganisms. These techniques were, however, time-consuming and poorly controlled and did not take into account variations in soil type and prevailing environmental conditions that largely control the final results.

Early studies for the detection of hydrocarbon-oxidising bacteria included examination for the formation of a bacterial pellicle, i.e. a film formed at the surface of a liquid medium inoculated with the soil samples, by methane or propane-oxidising organisms (Mogilevskii - cited by Kartsev et al "Geochemical methods of prospecting and exploration for petroleum and natural gas" Berkeley, Univ. Calif. Press, 1959). This technique is highly non-specific,

it is difficult to quantify the degree of enrichment and the method is time consuming to apply.

Other workers have used sprinkled (Davis et al, Appl. Microbiol. 7, 156-165, 1959) or clumped (Luchter, Nafta (Katowice) 9, 217-220, 1953) soil plating studies to isolate the hydrocarbon utilisers. These are also difficult to quantify and require a long incubation time as does soil dilution plating. Although plating-out methods have been used to enumerate more accurately the hydrocarbon utilising bacteria (Davis et al, 1959) they suffer from a poor efficiency of removal of bacteria from the soil.

Radioautography has also been employed by growing the bacterial colonies in the presence of labelled hydrocarbon and comparing numbers of colonies emitting beta-particles with total numbers of colonies isolated (Davies et al, 1959). Although this method does at least allow some form of standardisation, it nonetheless suffers from all the associated problems of dilution plating, not least of which is the lengthy incubation period. Such a prolonged period allows the adaption of microorganisms in soil from non-microseep areas to develop the capability of oxidising the hydrocarbon supplied in the experimental conditions.

Such criticism is applicable too to the methods of gas uptake used to quantify the rate of gaseous hydrocarbon oxidation in a culture medium supplemented with the soil sample (US-A-2665237, US-A-2861921, US-A-2875135, US-A-2880143, US-A-3174910 and Davis "Petroleum Microbiology" Elsevier, 1969). The specificity of such methods is also questionable.

Measurement of the oxidation of radioactive hydrocarbons by production of CO_2 was used by Davis et al (1959). However, the instrumentation employed is outdated and labour intensive. No attempt was made to reduce incubation time by optimisation of the physicochemical conditions so that incubation periods were of the same order of magnitude as previous studies. No microbiological

standardisation we carried out to control for environmental conditions and account for soil type, so that the system is open to spurious results.

We have now found that subsurface oil and gas deposits can be located much more readily from the distribution of hydrocarbon-oxidising microorganisms in the surface soil or sediment if the measure of microbial hydrocarbon-oxidising activity (i.e. the microbial activity due to microorganisms supported by the hydrocarbon gas concerned) is normalised with respect to the general level of microbial activity within the soil. This gives a standardised or normalised measure of hydrocarbon-oxidising activity regardless of any variations in environmental and soil conditions which control the general level of microbial activity. Further, we have also found that the hydrocarbon flux through soils, occurring over subsurface deposits, can be described and recognized much better by combining free soil gas measurements with measurements of microbial hydrocarbon-oxidising activity.

Accordingly, the present invention provides a method of assaying a plurality of soil samples collected at intervals across an exploration territory, which method comprises:

(i) determining from a first portion of a first sample a first value which represents a first microbial activity and which is attributable to microorganisms which metabolise a selected hydrocarbon gas;

(ii) determining from a second portion of the first sample a second value which represents a second microbial activity and which is attributable to a representative proportion of all microorganisms in the sample;

(iii) repeating steps (i) and (ii) for further samples; and

(iv) determining for each sample the first microbial activity relative to the second microbial activity from the said first and second values obtained for each sample.

In carrying out this method, soil samples are first collected at intervals across an exploration territory. The first value, which is determined in step (i), is attributable to microorganisms which metabolise a selected hydrocarbon gas. This gas may be a C_2-C_4 alkane such as ethane, propane, n-butane and isobutane. Preferably it is ethane. The first value may be determined using the selected hydrocarbon gas itself or such alcohol as would be derived from the microbial oxidation of the gas such as ethanol in the case of ethane. For one portion of each soil sample, therefore, the amount of a metabolite resulting from metabolism of a selected hydrocarbon gas or of a selected metabolite of such gas by microorganisms in the sample is determined. The metabolite determined is typically carbon dioxide.

For this purpose, a portion of each sample can be exposed to a radiolabelled hydrocarbon gas or to a radiolabelled alcohol derivative of a hydrocarbon gas for an incubation period. The time for which a portion of a sample is incubated with such substrate preferably corresponds approximately to the lag phase of microbial growth. In other words, it is the period prior to the increase of microbial biomass at the expense of substrate. Preferably, this period is as short as possible. This is in order to achieve rapid analysis. It is also to prevent microbes adapting to the substrate provided for the incubation. Further, it is for standardisation purposes.

The maximum initial rate at which a metabolite is produced in the lag phase may be determined. This is the initial peak rate and provides an index of the number of microorganisms exhibiting the particular metabolic activity concerned. The initial peak rate is determined to prevent the microbial population adapting to the substrate provided. Where it is hydrocarbon-oxidising microorganisms which are under analysis, this means that it is the initial peak rate of carbon dioxide production for which a value is

determined.

Preferably it is the substrate-induced maximal initial respiration rate at the end of the lag phase of microbial growth and the beginning of the log phase which is determined. This is the initial peak rate of substrate oxidation, and provides an index of the number of microorganisms exhibiting the particular metabolic activity concerned (Anderson and Domsch, Soil Biol. Biochem 10 215-211, 1978). To simplify this procedure we have found that, providing the period of incubation does not greatly exceed the average lag phase of soil microbial activity for a particular substrate, the quantity of radiolabelled carbon dioxide derived from the microbial oxidation of the substrate during the lag phase and earliest part of the log phase of growth, can be taken to be approximately proportional to the substrate-induced maximal initial respiration rate.

Determination of this quantity of carbon dioxide provides an index of the number of microorganisms exhibiting the particular metabolic activity concerned. It has been empirically determined for hydrocarbon-oxidising microorganisms in soils that this period should not exceed 16 hours. Limitation of the incubation period to this time prevents the microbial populations adapting to the substrate provided in or initiating log-phase growth and biomass increase.

We have therefore, substituted a single measurement of the quantity of carbon dioxide produced, in our standardised conditions, for the hour-to-hour measurements described previously (Anderson and Domsch) which would otherwise be required to obtain the substrate-induced maximal initial respiratory rates.

A measure is made of the amount of radiolabelled carbon dioxide gas produced by microorganisms oxidising the selected radiolabelled substrate. Preferably the radiolabelled substrate comprises radiolabelled ethane; or a radiolabelled substrate which is metabolised at an increased

rate by a hydrocarbon gas-adapted organism, typically an ethane-adapted organism, such as propane and butane; or an alcohol derivable from a hydrocarbon gas by microbial oxidation, such as ethanol from ethane. The radiolabelled substrate may therefore be an alcohol of a hydrocarbon gas. The measure of the amount of radiolabelled carbon dioxide gas which is produced over a short incubation period of time represents a measure of the specific microbial activity within the soil of microorganisms which feed from hydrocarbon gas.

Another portion of each soil sample is prepared. The amount of a metabolite, again typically carbon dioxide, is determined resulting from a metabolic activity possessed by a proportion of the microorganisms in a sample, the proportion being representative of all the microorganisms in the sample. This metabolic activity should therefore be possessed by a substantial proportion, typically a majority, of all microorganisms in the sample, such as the ability to oxidise glucose or another substrate.

A second portion of each sample can therefore be exposed to a radiolabelled substance (preferably radiolabelled glucose) which a substantial proportion of all microorganisms in the soil will oxidise to produce radiolabelled carbon dioxide. The amount of radiolabelled carbon dioxide so produced over a short period of time is measured.

The time for which a portion of a sample is incubated again preferably approximately corresponds to the lag phase of microbial growth. In other words, it is the period prior to the increase of microbial biomass at the expense of substrate. Preferably, this period is as short as possible. This is in order to achieve rapid analysis. It is also to prevent microbes adapting to the substrate provided for the incubation. Further, it is for standardisation purposes.

The maximum initial rate at which a metabolite such as carbon dioxide may be produced in the lag phase is

determined. This is the initial peak rate and provides an index of the number of microorganisms exhibiting the particular metabolic activity concerned. The initial peak rate is determined to prevent the microbial population adapting to the substrate provided. Where it is glucose-oxidising microorganisms which are under analysis, this means that it is the initial peak rate of carbon dioxide production for which a value is determined.

Preferably it is the substrate-induced maximal initial respiration rate at the end of the lag phase of microbial growth and the beginning of the log phase which is determined. This is the initial peak rate of substrate oxidation, and provides an index of the number of microorganisms exhibiting the particular metabolic activity concerned (Anderson and Domsch). As indicated above, we have found that, providing the period of incubation does not greatly exceed the average lag phase of soil microbial activity for a particular substrate, the quantity of radiolabelled carbon dioxide derived from the microbial oxidation of the substrate during the lag phase and earliest part of the log phase of growth, can be taken to be approximately proportional to the substrate-induced maximal initial respiration rate.

Determination of this quantity of carbon dioxide provides an index of the number of microorganisms exhibiting the particular metabolic activity concerned. It has been empirically determined that, for glucose-oxidising organisms, this period should not exceed 6 hours. Limitation of the incubation period to this time prevents the initiation of log-phase growth and biomass increase.

We have, therefore, substituted a single measurement of the quantity of carbon dioxide produced, in our standardised conditions, for the hour-to-hour measurements described previously (Anderson and Domsch) which would otherwise be required to obtain the substrate-induced maximal initial respiratory rates.

Preferably then an index is determined for each

sample, being a measure of the amount of radiolabelled carbon dioxide produced by the sample exposed to radiolabelled hydrocarbon gas or radiolabelled alcohol of a type which results from the microbial oxidation of a hydrocarbon gas, typically ethanol, propanol, butanol or isobutanol, relative to the amount of radiolabelled carbon dioxide produced by the sample exposed to radiolabelled glucose. Other substances may be used instead of glucose, particularly salts of acetic or succinic acid.

The values obtained in step (iv) may then be plotted against distance across the exploration territory. A plot of measurements representing or dependent upon the microbial activity of microbes supported by the selected hydrocarbon gas (e.g. ethane) can indicate one or more zones of higher relative exposure to the selected hydrocarbon gas than other zones of the exploration territory.

It is possible to measure the concentrations of hydrocarbon gases freely contained in the soil at sample locations across the exploration territory. A plot against distance of measurements representing free hydrocarbon concentrations in the soil can indicate anomalous zones of higher concentrations of hydrocarbon gas. We have found that if the results of a microbial analysis and the results of a free gas analysis are combined, a plot is obtained which defines more clearly anomalous zones of intense and continuous hydrocarbon flux to the surface. Such zones can then be subjected to further exploration efforts.

The invention therefore also provides a method of assaying a plurality of sites across an exploration territory for subsurface oil or gas deposits, which method comprises:

(a) assaying soil samples taken at each site by the method above, thereby determining for each site the microbial activity attributable to microorganisms which metabolise a selected hydrocarbon gas relative to the microbial activity attributable to a substantial proportion of all microorganisms;

(b) determining the free gas concentration at each site of a selected hydrocarbon gas; and

(c) for each site, combining the determination made in step (a) and the determination made in step (b).

In order to combine the two measurements for each sampling site resulting from steps (a) and (b), preferably the two measurements are multiplied together. The thus factored data can then be plotted against distance over the exploration territory. In determining the concentration of free hydrocarbon gas, the concentrations of any one or more hydrocarbon gases (particularly ethane, propane, n-butane and isobutane) in each sample can be measured. If the concentrations of more than one such gas are measured, preferably they are added together to give a resultant free hydrocarbon gas concentration measurement for combining with the corresponding microbial activity measurement.

In more detail, an exploration for subsurface oil or gas deposits comprises one and optionally two sample collection and analysis procedures. One, which may be optional, involves determining the concentration of at least one free hydrocarbon in the soil at successive sampling sites. The other involves determining the microbial activity at the identical sites of microorganisms which are supported by a selected hydrocarbon gas. The results from these two procedures can then be combined together, if required.

In the one procedure, the concentration of one or more hydrocarbon gases in the free gas in the soil is determined at sites at regular intervals across the territory being explored. Thus, at each of these sites, a hole is drilled into the soil to a predetermined depth and a hollow probe is then inserted into this hole, the probe having an apertured bottom end which allows soil gases to flow in and then up inside the probe to a gas-tight syringe. The syringe is used to take a predetermined volume of gas, which is then injected into a sealed, pre-evacuated vial.

The concentration of the selected hydrocarbon gas or gases in each sample is generally determined using a gas chromatograph, for example as ppm.

The data obtained by this procedure are then used to provide a plot showing the variations in the concentration of the selected hydrocarbon gas or gases with distance across the explored territory. The hydrocarbon gas is generally one or more C_2-C_4 alkane such as ethane, propane, n-butane and isobutane. Preferably the concentrations in each sample of ethane, propane, n-butane and isobutane are determined and added together to provide a resultant free hydrocarbon gas concentration measurement.

In the other procedure, samples of soil are taken at sites at regular intervals across the territory being explored. At each site, the sample of soil is taken at a depth of 15cm to 30cm (6 inches to one foot) or otherwise below the prevailing root zone and placed into a sample container which is then immediately sealed and stored at a predetermined temperature, e.g. 4°C, until analysis.

A predetermined quantity (e.g. 10 gms) of each soil sample is ground with a predetermined quantity of soil conditioner, e.g. perlite. Equal parts (e.g. 5 gms) of the sample are then placed in two vials: into the first vial a predetermined quantity of nutrient solution is added and the vial is sealed, while into the second vial a predetermined quantity is added of a nutrient solution which includes radiolabelled glucose of known radioactive content and the vial is sealed. Further into the first vial a predetermined volume of a radiolabelled hydrocarbon preferably ethane, or a predetermined amount (volume or weight) of a radiolabelled alcohol derivative of a hydrocarbon gas, of a known radioactive content is introduced. Preferably both the glucose and the hydrocarbon or alcohol are radiolabelled with carbon 14.

Next the two vials from each sample are incubated at a predetermined temperature for predetermined time periods. The incubation period preferably corresponds to about the lag phase of microbial growth. For example, the glucose-containing vials may be incubated at 30°C for 6 hours. The hydrocarbon gas- or hydrocarbon gas-derived alcohol containing vials may be incubated at 30°C for 16 hours. At the end of their respective incubation periods, the biological activity in the vials is stopped, e.g. by autoclaving.

During the period of incubation, in each hydrocarbon gas- or hydrocarbon-derived alcohol containing vial any microorganisms which feed from the gas or alcohol will have oxidised some of the radiolabelled gas or alcohol to give off radiolabelled carbon dioxide gas. The amount of radiolabelled carbon dioxide will be proportional to the microbial activity of microorganisms present in that sample which oxidise the selected hydrocarbon gas. Similarly in each glucose vial, during the period of incubation a substantial proportion of all microorganisms present in the soil sample will oxidise the radiolabelled glucose to give off radiolabelled carbon dioxide gas. Thus the amount of radiolabelled carbon dioxide which accumulates in the headspace of the glucose vial will be proportional to the microbial activity of a substantial proportion of all microorganisms present in the sample.

The amount of radiolabelled carbon dioxide in the headspace of each of the hydrocarbon gas or hydrocarbon gas-derived alcohol and glucose sample vials is determined using a gas chromatograph and an appropriate radioactivity detector as follows. A batch of vials is loaded into an automatic headspace sampler linked to a gas chromatograph. In the headspace sampler successive vials are moved in turn

to a sampling station, at which a needle is lowered to pierce the rubber septum of the vial.

An inert gas is injected through the needle into the vial to pressurize its headspace. Then the source of inert gas is closed off, and the headspace is connected to the gas chromatograph. The gas in the vial headspace flows to the gas chromatograph, where the various components in the headspace gas are separated. The gas chromatograph detector gives a signal representing the mass of the respective components of gas, and the mass of these components is automatically recorded on a computer linked to the gas chromatograph.

The successive gas components then pass to the radioactivity detector, which serves to measure the amounts of radioactivity in the carbon dioxide and, where appropriate, the selected hydrocarbon gas components and these amounts are automatically recorded on the computer. Before the headspace of the next sample is communicated to the gas chromatograph and radioactivity detector, these are flushed out by passing the inert gas for a predetermined period.

The amounts of radiolabelled carbon dioxide produced in the glucose and hydrocarbon gas or hydrocarbon gas-derived alcohol vials from each sample are determined, and then compared. The first value obtained in step (i) is expressed as a proportion of the second value obtained in step (ii) for paired first and second portions of a sample. The first value may be expressed as a ratio, fraction or percentage of the second value. For example, for each soil sample collected an ethane index can be calculated as follows:

$$\text{ethane index} = \frac{^{14}CO_2 \text{ from ethane vial} \times 100}{^{14}CO_2 \text{ from glucose vial}}$$

Choosing the ethane index as an example, for each soil sample a measure is obtained which represents the microbial activity of the ethane-supported microorganisms relative to the activity of a high proportion of the total microorganisms present in the sample. From the ethane indices determined for all the soil samples collected from the exploration territory, a plot can be made of ethane index against distance across the exploration territory.

The free gas plot described previously identifies areas of higher concentrations of free hydrocarbon gas in the soil. Similarly, the corresponding ethane index plot identifies areas of higher ethane index. We have found that by combining the free gas and ethane index data for the corresponding soil samples, a plot is obtained which defines more clearly areas of intense and continuous hydrocarbon flux to the surface. Preferably the measurement of free gas concentration in respect of each sample site of the exploration territory is multiplied by the ethane index determined in respect of the same sample site.

While carbon 14 radiolabelled substrates may be used, it is also possible to use the carbon isotope of atomic weight 13 to label the substrates. A mass spectrometer is then used at the output of the gas chromatograph to differentiate between carbon dioxide with carbon of the normal isotope atomic weight 12 and the carbon dioxide labelled with carbon 13, and to measure the amount of the latter. An index based on carbon 13 results may then be generated. Other labelled substances, such as alkali metal, alkaline earth metal and ammonium salts of acetic or of succinic acid, may be used instead of glucose.

The following Examples illustrate the invention. In the accompanying drawings:

Figures 1 to 3 show plots of data across field A;
Figures 4 to 6 show plots of data across field B;
Figures 7 to 9 show plots of data across field C; and
Figure 10 shows a plot of data across field D.

EXAMPLE 1

Sample collection

Corresponding soil and gas samples were taken at various sampling points across the exploration area of interest. Samples were routinely taken at 0.2 km or 0.4 km (1/8 or 1/4 mile) intervals. At each site, about 250 grams of soil sample was taken at a depth of 15 cm to 30 cm (six inches to one foot) (or below the root zone) and placed in a self-sealing plastic bag, which was immediately placed in an ice chest maintained at about 4°C. All soil samples were stored at 4°C until analysed.

At the same sampling point, a hole of 14.29 mm (9/16 inch) diameter was drilled 1.2 m into the soil. A gas sampling probe of identical dimensions was then inserted into the hole. The probe consisted of a hollow steel rod punctured at the bottom to allow soil gases to flow through the probe to the top into a gas tight syringe. The dead volume of the probe was 30ml. The first 10ml of gas was taken and discarded. 125ml of gas was then taken with the syringe and injected into a septum-sealed, pre-evacuated 120ml glass vial.

Sample processing and analysis

1. Soil hydrocarbon determination

A conventional gas chromatograph equipped with a flame ionization detector and an inert Al_2O_3/KCl capillary column (50m in length) was used to analyse and quantify hydrocarbons in soil gas. A backflush system limited the

analyses to a predetermined molecular range and avoided any column contamination with higher molecular weight hydrocarbons or associated gases. Completely inert columns and ultrapure operating gases were used. These allowed the determination of hydrocarbon gases in the sub-ppm range with a precision of better than 5%. The detection limit was in the 10-20ppb range. The concentrations of ethane, propane and n-butane for each soil gas sample were summed.

2. Soil hydrocarbon-oxidizing activity determination

9 grams of each soil sample was ground with 1 gram of perlite. 5 grams of the ground sample were placed into each of two Perkin Elmer HS100 autosampler vials. 1ml of nutrient solution A was added to the first vial, which was then sealed with a rubber septum. 1ml of nutrient solution B, containing 3.5 μCi ^{14}C-radiolabelled glucose (specific activity 2.9 μCi/μmole) was added to the second vial, which was then sealed.

Approximately 100 μmoles of ^{14}C-radiolabelled ethane (specific activity 0.025 μCi/μmole) was then added to the first vial. The glucose-containing vials were incubated at 30°C for 6 hours and the ethane-containing vials were incubated at 30°C for 16 hours. At the end of the respective incubation periods, microbial activity was stopped by autoclaving the vials.

Nutrient Solution A

KH_2PO_4	7.5g
NH_4NO_3	7.5g
$MgSO_4.7H_2O$	3.0g
$CaCl_2.6H_2O$	0.15g
$FeSO_4.7H_2O$	0.075g

Distilled water was added to 1 litre and the pH was adjusted to 7 with sodium hydroxide.

Nutrient Solution B

As for nutrient solution A but supplemented with 25 grams per litre of glucose.

The amount of ^{14}C-radiolabelled carbon dioxide produced by each soil sample was then determined using a combined headspace sampler-gas chromatograph linked to gas proportional counter (GPC). The gas chromatograph was equipped with a thermal conductivity detector (TCD) and a back-flush system. The latter prevented radioactive ethane from passing to the TCD and the GPC. The GPC was linked directly to the TCD vent pipe and used 90% Argon/10% Methane as the quench gas. The gas chromatograph was fitted with a 5m stainless steel column containing Porapak R. Helium was used the carrier gas.

The amount of radioactivity present in the carbon dioxide component of the headspace gas was determined by the GPC after gas chromatographic separation from other gases present in the vial. The GPC was linked to a computing integrator, which automatically calculated the amount of radioactivity present in each component gas presented to the GPC. Each component gas was recognised by its unique retention time and labelled appropriately.

For each soil sample, the amount of radioactive carbon dioxide generated from added radiolabelled glucose and ethane was determined. An ethane index for each soil was then calculated as follows:

$$\frac{^{14}C\text{-}CO_2 \text{ from ethane} \times 100}{^{14}C\text{-}CO_2 \text{ from glucose}}$$

Result interpretation

The ethane index and free gas results were then plotted against their respective sample points or distance across the exploration area.. Plots achieved after processing samples taken over known oil and gas fields are presented in Figure 1-6. The results for the microbial and free gas surveys over fields A and B (Figs. 1, 2, 4 and 5) clearly demonstrate that anomalously high values can indicate the presence of these fields. In contrast, a microbial and free gas survey over field C (Figs. 7 and 8) did not provide a clear indication of the field's presence.

Factoring the microbial and free gas from the same survey had the effect of reducing the background noise signal and more clearly outlining the presence of the field. Factoring was accomplished by simply multiplying the ethane index value by the free gas value. The results for fields A and B (Figs. 3 and 6) demonstrate that factored data confirmed the presence of the fields previously identified by microbial or free gas data alone. However, factored data can demonstrate the presence of a field not recognised by microbial or free gas data alone. This is clearly demonstrated with field C (Fig. 9). A further example of the value of factored data in identifying the presence of an oil and gas field is presented in Fig. 10. The background noise signal has been reduced to a minimum and the presence of the field is clearly indicated by the high values over the field.

Example 2: Oil hydrocarbon-oxidizing activities using alternative substrates

To demonstrate the feasibility of using the oxidation of other hydrocarbons as a marker for oil- and gas-derived soil chemical constituents, the following experiment was undertaken.

A 200g sample of a garden topsoil was placed into a 1L glass jar and the jar sealed. The soil was then incubated at 25°C in the presence of ethane, a small quantity of unlabelled ethane gas being first injected into the jar via a rubber septum to give a final concentration of ethane in air of 10%. After 2 months incubation in a regularly replenished ethane/air atmosphere, this 'ethane-adapted' soil was diluted (5:7) with fresh garden soil and tested for its ability to oxidize both ethane and a range of associated hydrocarbons. The latter included the gases propane and n-butane, and the alcohols ethanol, propan-1-ol and butan-1-ol. In addition, fresh soil from the same site (without prior mixing with the ethane-adapted soil), was tested for similar criteria.

Samples of both soils were treated in a similar fashion to those for standard ethane analyses, except that as well as ^{14}C-labelled ethane, vials containing either ^{14}C-labelled propane, butane, ethanol, propan-1-ol or butan-1-ol were also incubated, and gas samples from the vials assessed for the quantities of $^{14}CO_2$ present after incubation. Hydrocarbon gas samples were incubated for 16 hours, and the alcohols for 6 hours. Standard ^{14}C-glucose samples were also prepared and sampled after 6 hours incubation. Ten replicate samples were prepared, incubated and analysed for each substrate in each soil.

Results for the quantities of $^{14}CO_2$ produced from each substrate were expressed as a percentage of those from the glucose treatments, to produce an index for each hydrocarbon substrate. The results were as follows.

ADAPTED SOIL	Mean $^{14}CO_2$ produced ± standard deviation			Index
Glucose	467.7	±	42.2	-
Ethane	4317.6	±	843.8	923.2
Propane	1234.7	±	38.5	263.9
N-Butane	2933.6	±	286.9	627.2
Ethanol	2276.8	±	117.2	486.8
Propan-1-ol	237.1	±	26.2	50.7
Butan-1-ol	33.0	±	20.2	7.1
NON-ADAPTED SOIL				
Glucose	312.4	±	61.5	-
Ethane	29.0	±	10.0	9.3
Propane	40.6	±	13.3	12.9
N-Butane	204.6	±	23.9	65.3
Ethanol	405.8	±	41.3	129.9
Propan-1-ol	123.6	±	15.2	39.6
Butan-1-ol	39.2	±	27.8	12.5

Results show the clear difference between the two soils in terms of their microbial oxidizing activities, and the markedly higher indices obtained with the ethane-adapted soil for all but the butan-1-ol. Propane, butane and ethanol appear to be particularly suitable alternative substrates to ethane for exploratory work.

CLAIMS

1. A method of assaying a plurality of soil samples collected at intervals across an exploration territory for subsurface oil or gas deposits, which method comprises:

(i) determining from a first portion of a first sample a first value which represents a first microbial activity and which is attributable to microorganisms which metabolise a selected hydrocarbon gas;

(ii) determining from a second portion of the first sample a second value which represents a second microbial activity and which is attributable to a representative proportion of all microorganisms in the sample;

(iii) repeating steps (i) and (ii) for further samples; and

(iv) determining for each sample the first microbial activity relative to the second microbial activity from the said first and second values obtained for each sample.

2. A method according to claim 1, wherein the first portion of the or each sample is incubated with a radiolabelled hydrocarbon gas or a radiolabelled alcohol derivable from a hydrocarbon gas by microbial oxidation.

3. A method according to claim 2, wherein the first portion of the or each sample is incubated with radiolabelled ethane.

4. A method according to claim 1, wherein the second portion of the or each sample is incubated with radiolabelled glucose or with an alkali metal, alkaline earth metal or ammonium salt of radiolabelled acetic or succinic acid.

5. A method according to claim 1, wherein the maximum initial rate at which a metabolite is produced in

the periods approximately equivalent to the lag phase of microbial growth is determined in steps (i) and (ii).

6. A method according to claim 1, wherein the amount of radiolabelled carbon dioxide produced is measured in steps (i) and (ii).

7. A method according to claim 1, wherein the first value from step (i) is expressed as a ratio, fraction or percentage of the second value from step (ii).

8. A method according to claim 1, further comprising determining the concentration of free hydrocarbon gas at each site at which a soil sample is taken and, for each site, combining the determination made in step (iv) and the determination of the concentration of free hydrocarbon gas.

9. A method according to claim 8, wherein the determination made in step (iv) is multiplied with the determination of free hydrocarbon gas concentration.

10. A method according to claim 1, further comprising plotting the results obtained against distance across the exploration territory.

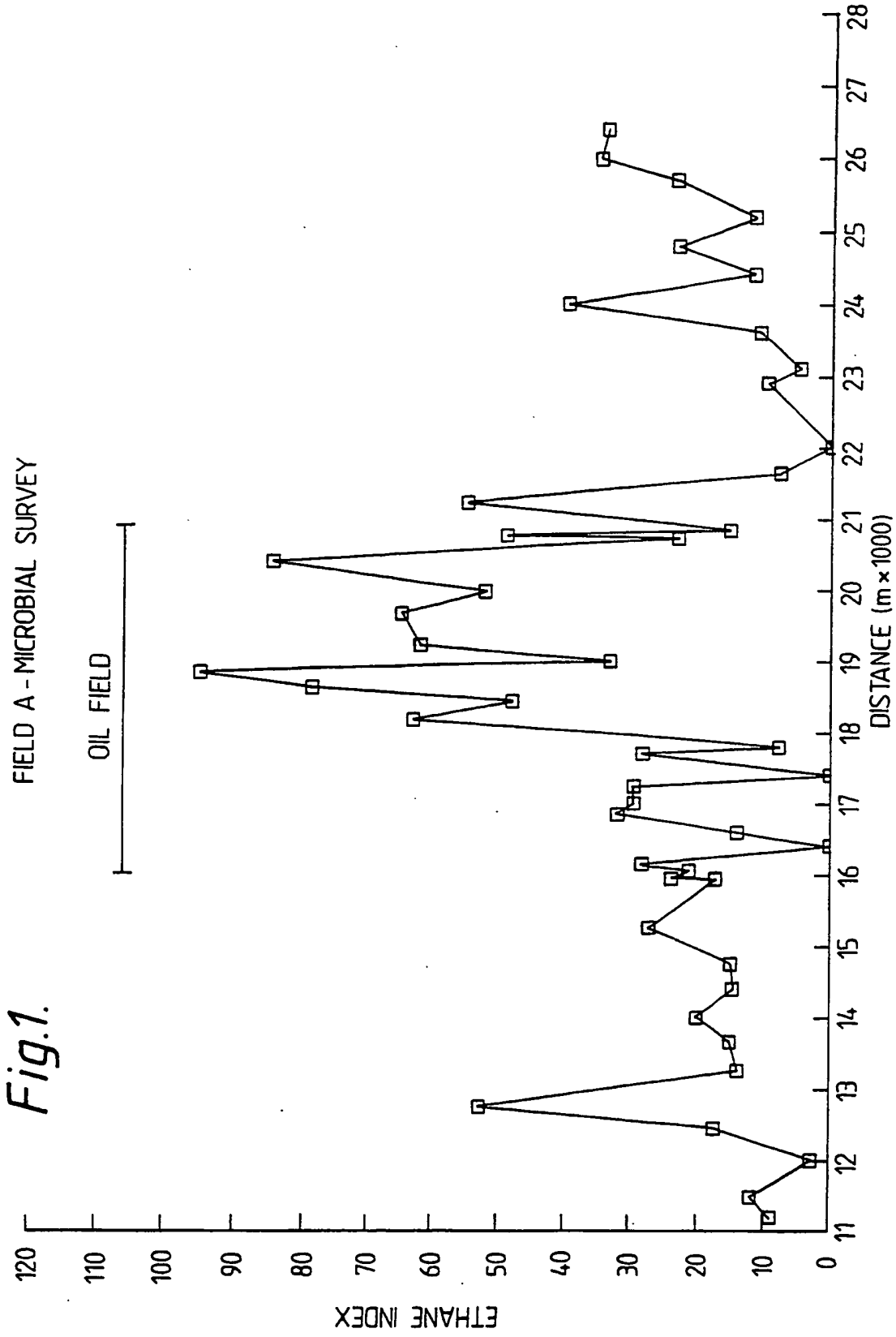
FIELD A - MICROBIAL SURVEY
OIL FIELD
ETHANE INDEX
DISTANCE (m x 1000)
0
10
20
30
40
50
60
70
80
90
100
110
120
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

Fig.1.

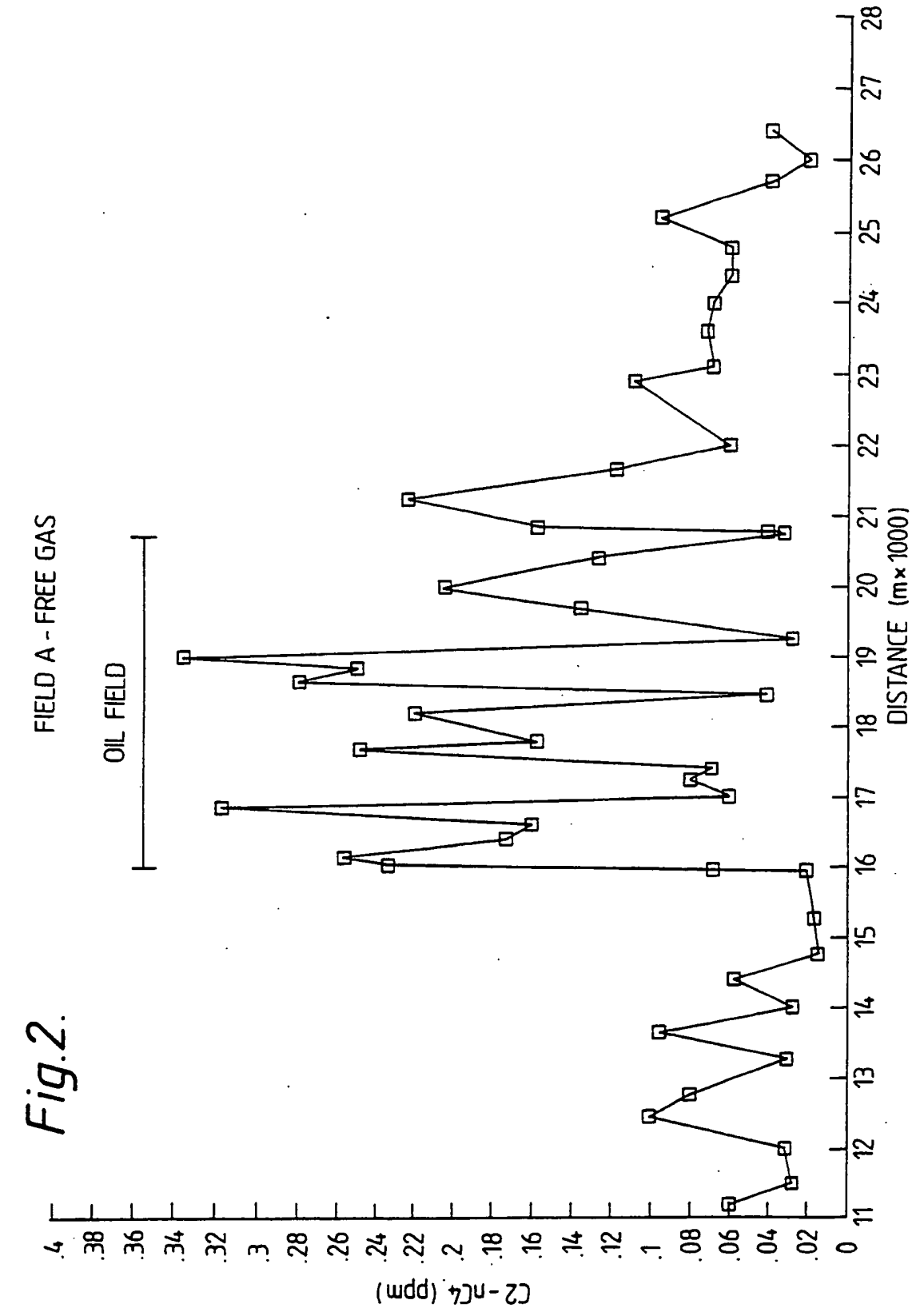
FIELD A - FREE GAS
OIL FIELD
DISTANCE (m×1000)
C2 - nC4 (ppm)
0
.02
.04
.06
.08
.1
.12
.14
.16
.18
.2
.22
.24
.26
.28
.3
.32
.34
.36
.38
.4
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

Fig.2.

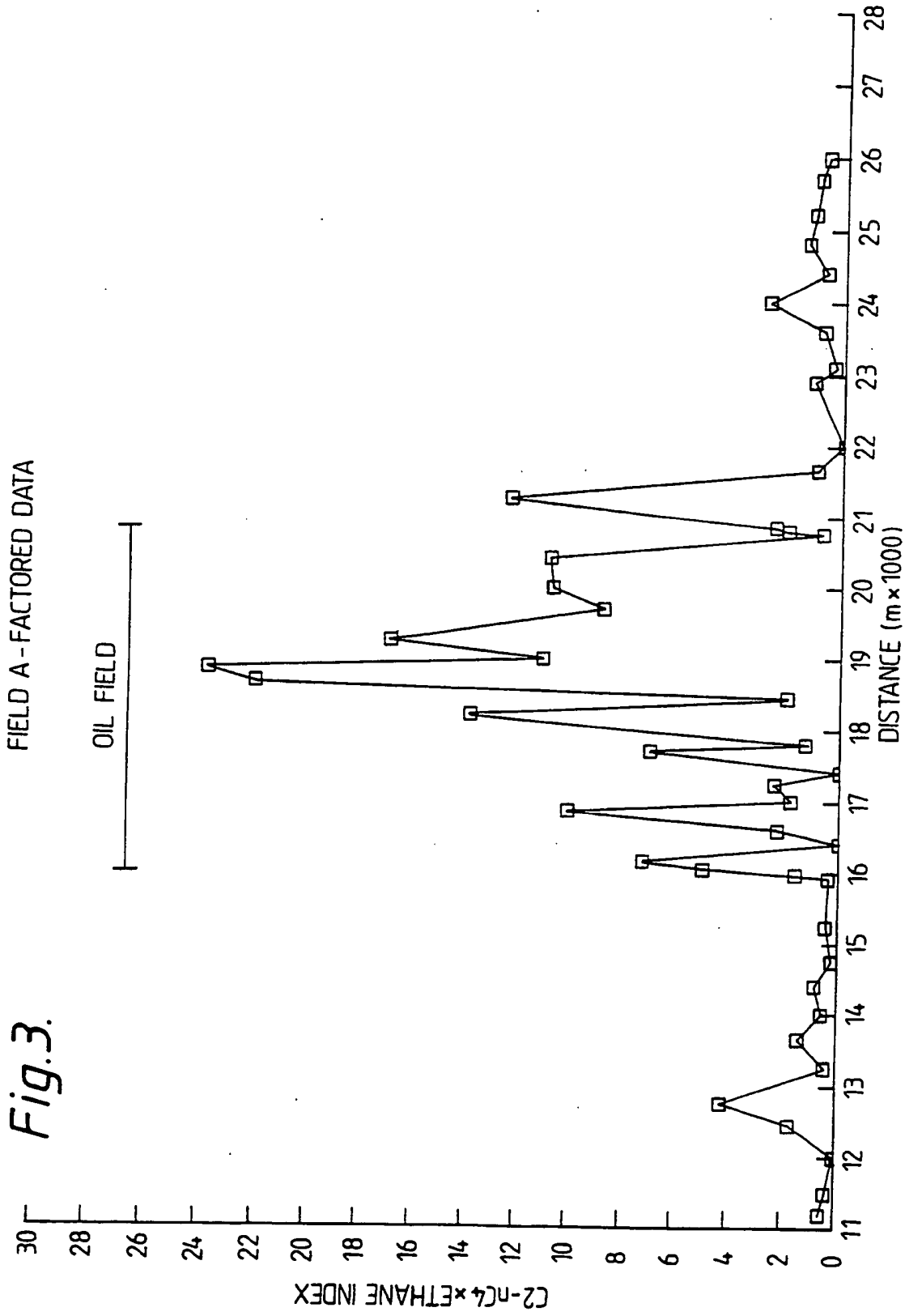
FIELD A - FACTORED DATA
OIL FIELD
C2-nC4 × ETHANE INDEX
DISTANCE (m × 1000)
0
2
4
6
8
10
12
14
16
18
20
22
24
26
28
30
11
12
13
14
15
16
17
18
19
20
21
22
23
24
25
26
27
28

Fig.3.

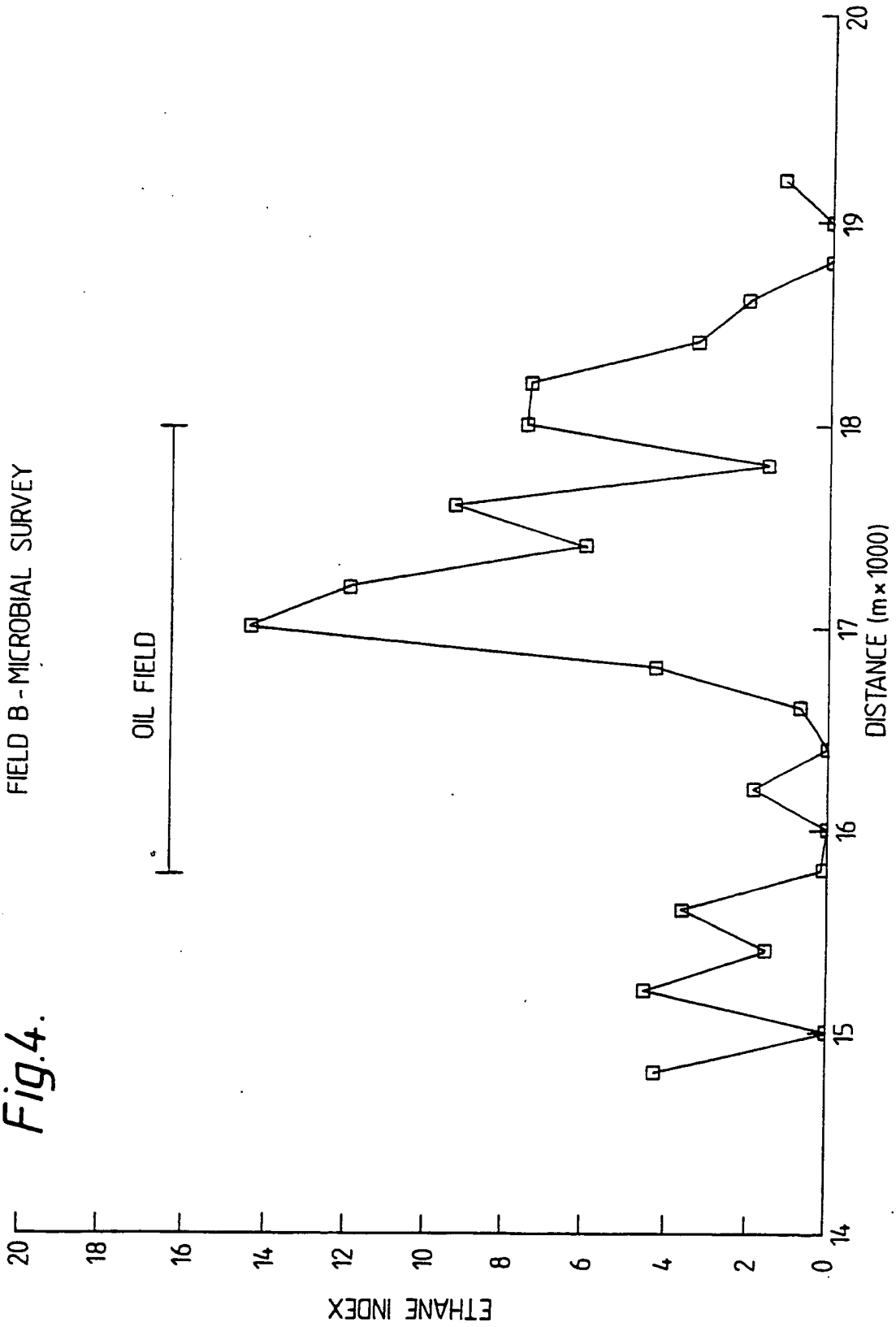
FIELD B - MICROBIAL SURVEY
OIL FIELD
ETHANE INDEX
0
2
4
6
8
10
12
14
16
18
20
DISTANCE (m × 1000)
14
15
16
17
18
19
20

Fig.4.

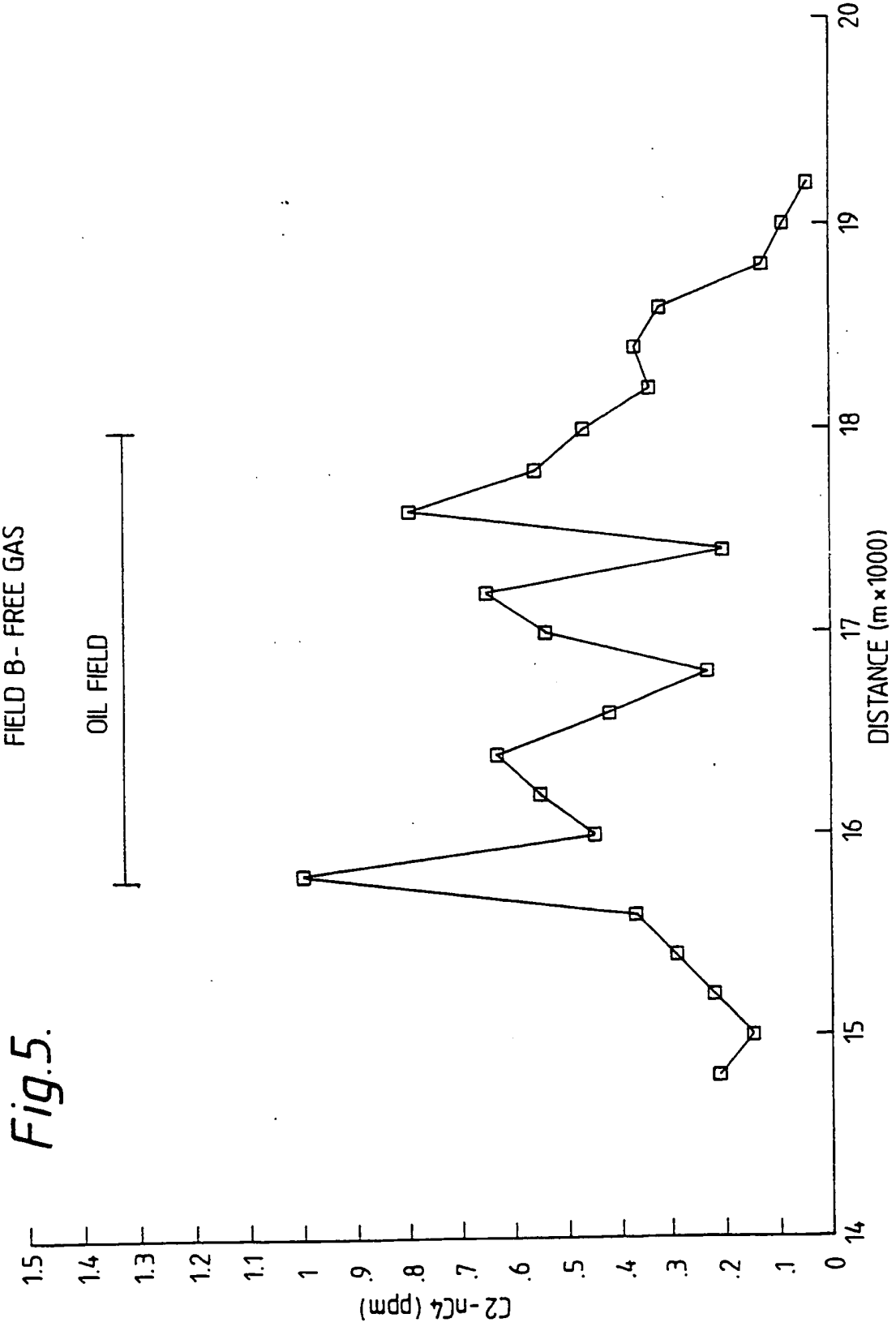
FIELD B- FREE GAS
OIL FIELD
C2-nC4 (ppm)
1.5
1.4
1.3
1.2
1.1
1
.9
.8
.7
.6
.5
.4
.3
.2
.1
0
14
15
16
17
18
19
20
DISTANCE (m x 1000)

Fig.5.

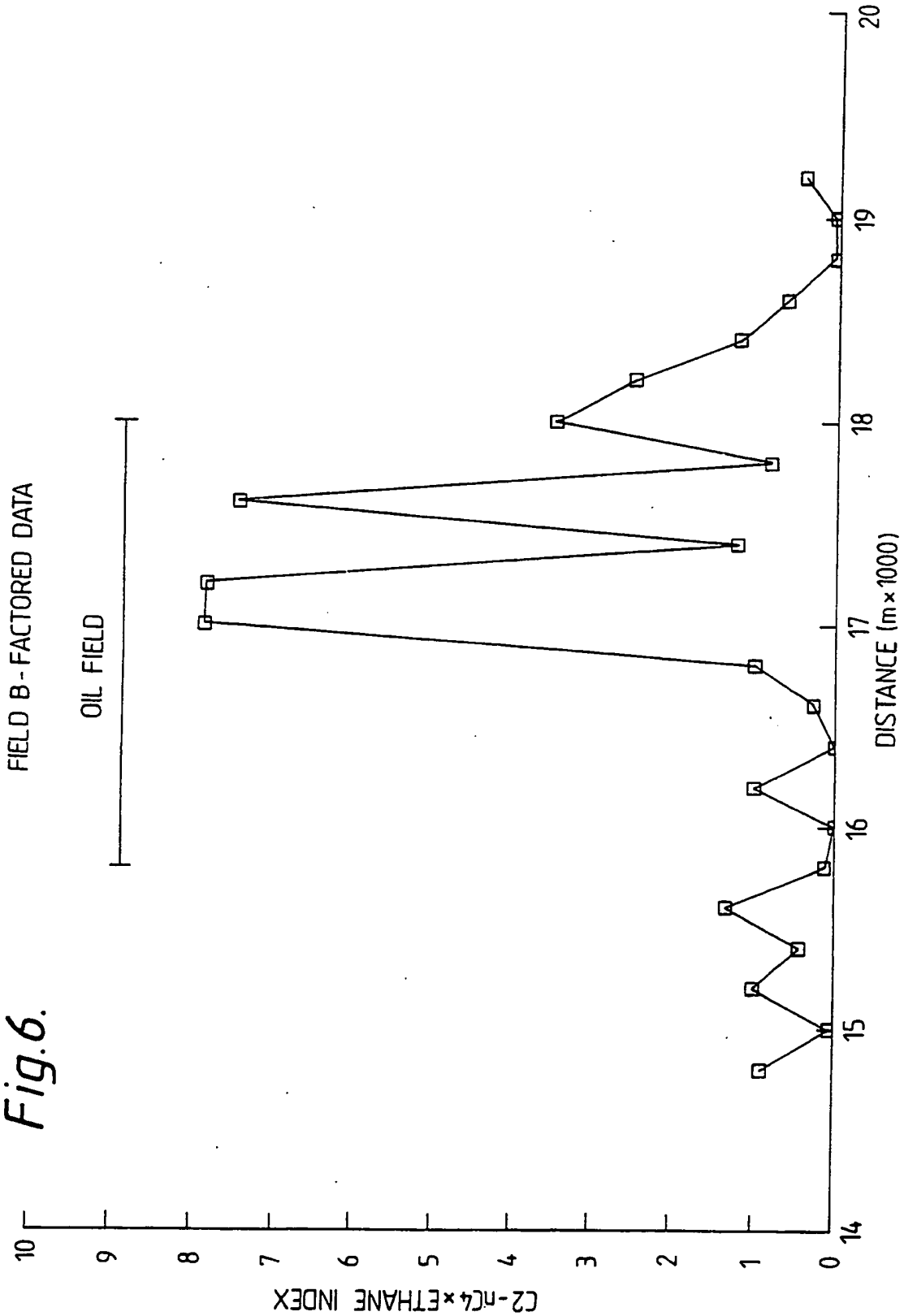
FIELD B - FACTORED DATA
OIL FIELD
C2-nC4 × ETHANE INDEX
0
1
2
3
4
5
6
7
8
9
10
14
15
16
17
18
19
20
DISTANCE (m × 1000)

Fig.6.

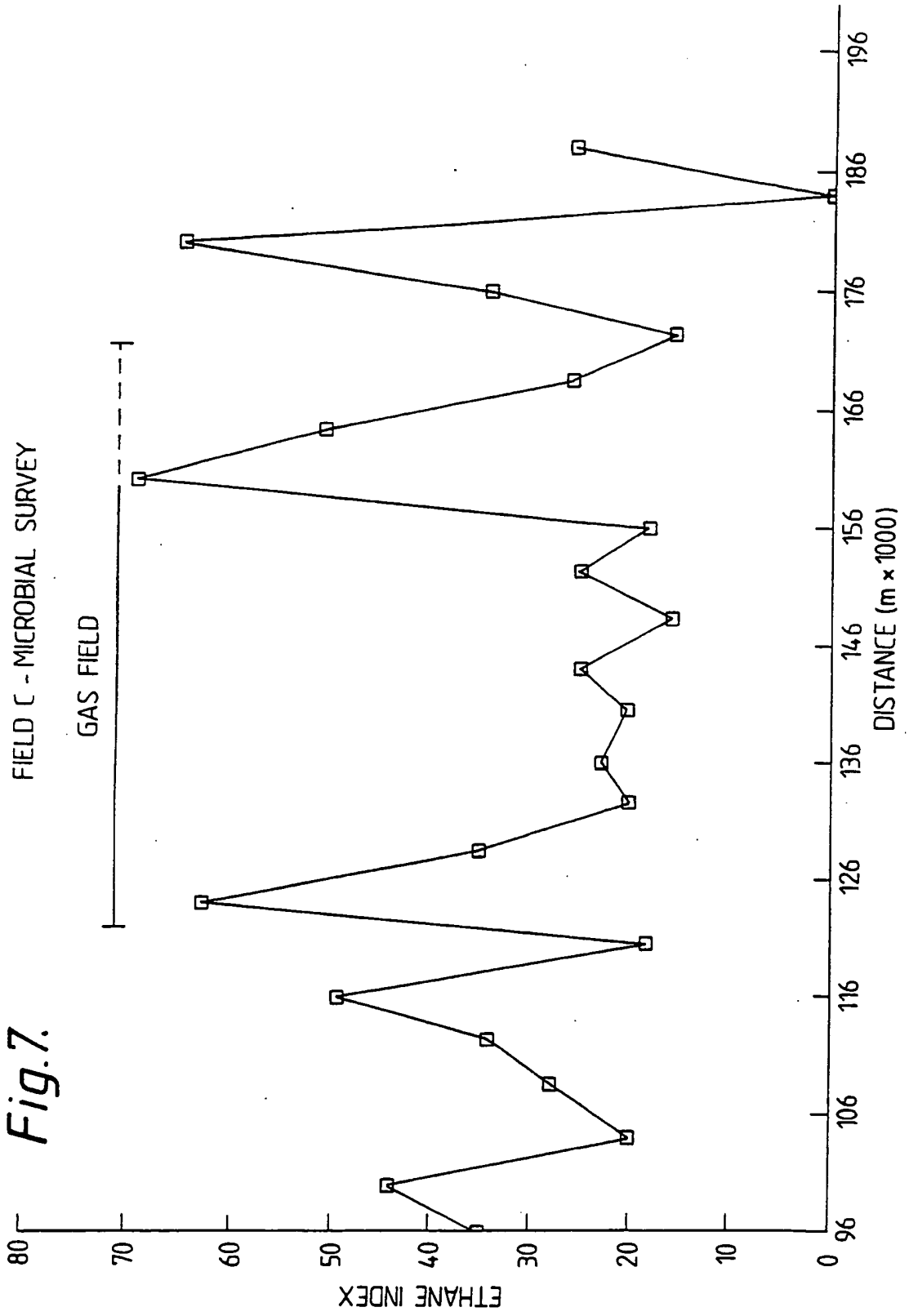
FIELD C - MICROBIAL SURVEY
GAS FIELD
ETHANE INDEX
80
70
60
50
40
30
20
10
0
96
106
116
126
136
146
156
166
176
186
196
DISTANCE (m × 1000)

Fig.7.

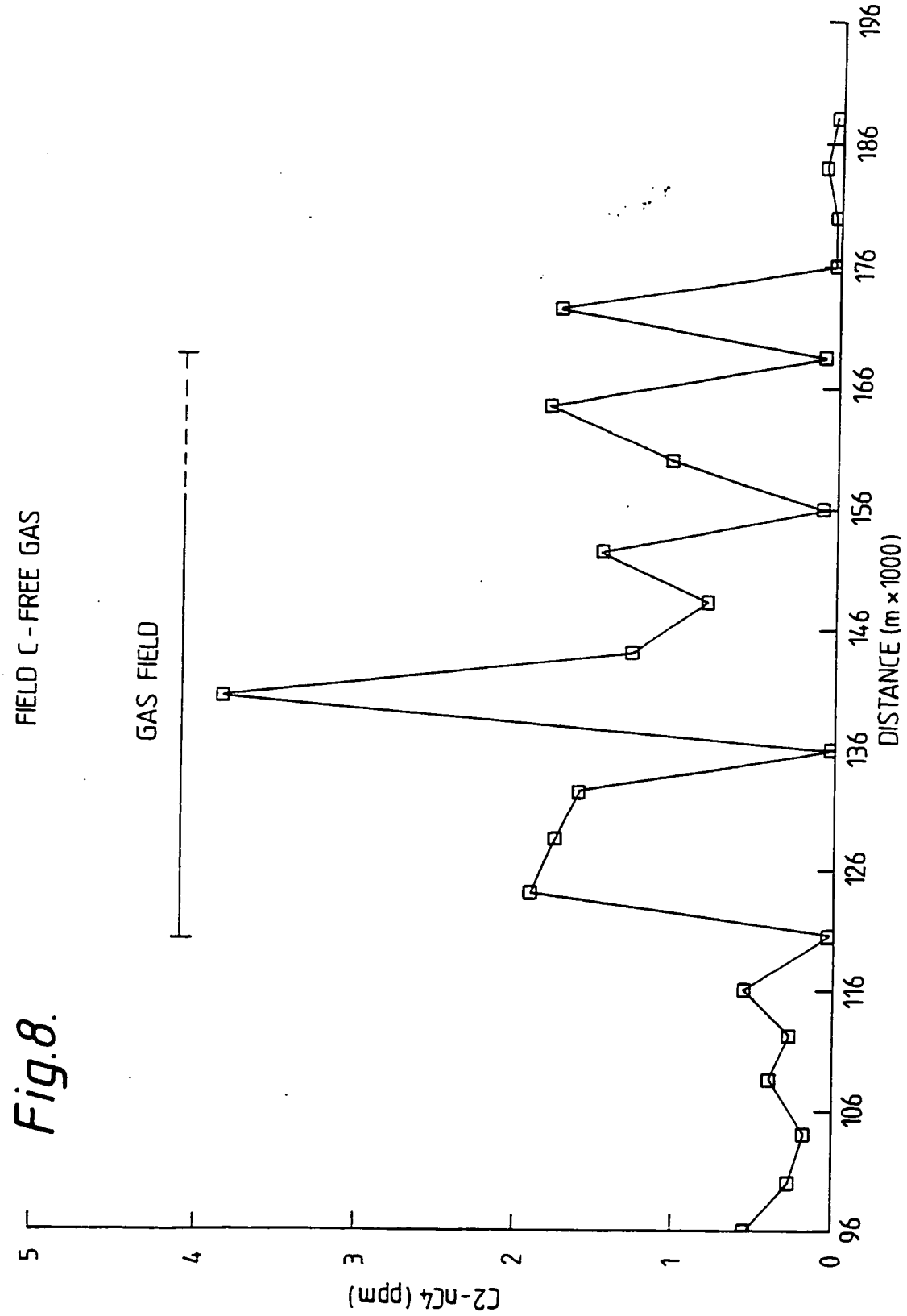
FIELD C - FREE GAS
GAS FIELD
C2-nC4 (ppm)
0
1
2
3
4
5
96
106
116
126
136
146
156
166
176
186
196
DISTANCE (m x 1000)

Fig.8.

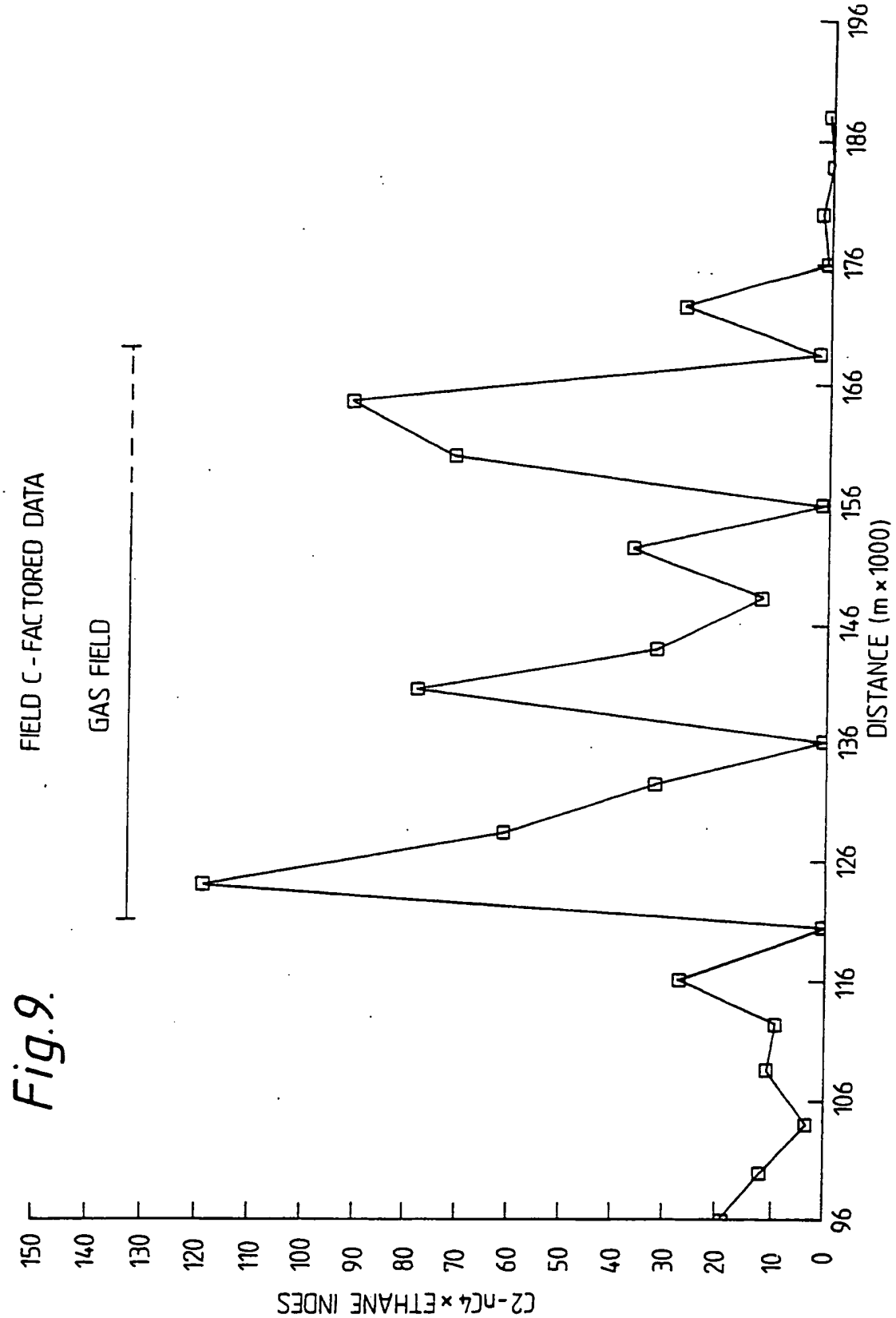
FIELD C - FACTORED DATA
GAS FIELD
C2-nC4 × ETHANE INDES
DISTANCE (m × 1000)
0
10
20
30
40
50
60
70
80
90
100
110
120
130
140
150
96
106
116
126
136
146
156
166
176
186
196

Fig.9.

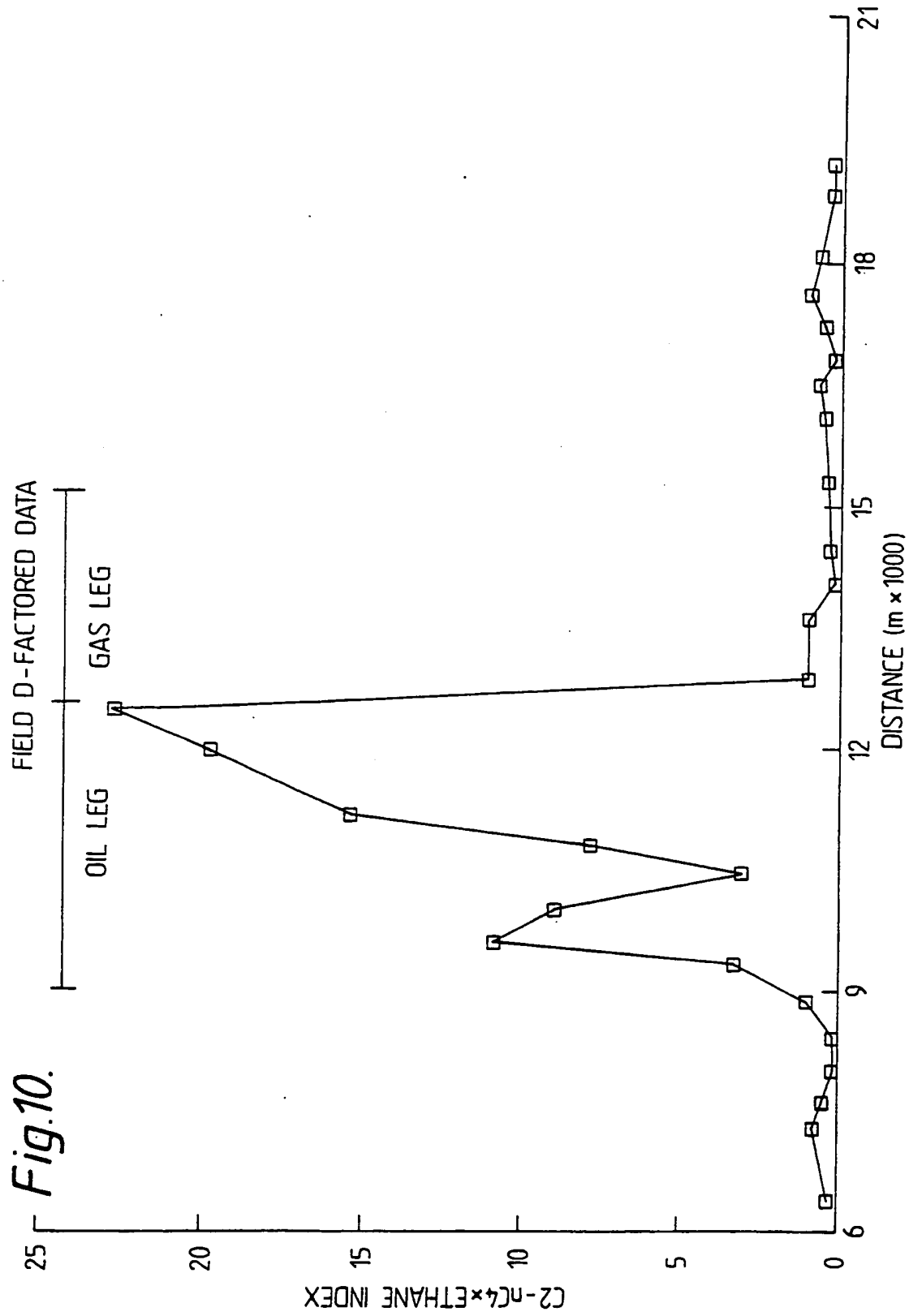
FIELD D-FACTORED DATA
OIL LEG
GAS LEG
C2-nC4×ETHANE INDEX
DISTANCE (m×1000)
0
5
10
15
20
25
6
9
12
15
18
21

Fig.10.

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 90/01184

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: C 12 Q 1/64, G 01 V 9/00

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	C 12 Q, G 01 V

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
A,P	WO, A, 90/02816 (GENENCOR, INC.) 22 March 1990 see pages 1-12	1
A	Appl. Microbiol., volume 7, 1959, J.B. Davis et al.: "Areal contrasts in the abundance of hydrocarbon oxidizing microbes in soils", pages 156-165 see pages 156-160 cited in the application	1-3
A	US, A, 3174910 (D.O. HITZMAN) 23 March 1965 see the whole document cited in the application	1

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
17th September 1990	15 OCT. 1990
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	MISS T. TAZELAAR

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9001184 SA 38816

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 05/10/90
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A- 9002816	22-03-90	AU-A- 4318389	02-04-90
US-A- 3174910		None	